REMARKS

The current application is a divisional of the 09/333,213 application, now issued as U.S. Patent No. 6,548,653 ("the '653 patent"). Pursuant to the MPEP, the claims added above represent separate and patentably distinct claims from those found in the allowed claims of the '653 patent enabled by the original filing.

Applicant must also note that they are prepared to use all possible efforts to work with the Examiner to remove all the rejections to the remaining claims. The Applicants believe that the amendments which have been made during the prosecution history of this application, along with the extensive nature of this response, serve to put all the pending claims in condition for allowance.

Claims 1-26, and 46-68 are currently pending. Claims 1, 2, 18-19, 23-24, 26, 46 and 47 are amended herein. No claims are canceled herein, other than those non-elected as a result of the Examiner's Restriction. Claims 48-68 are added herein. No new matter is added herein.

Early and favorable review of the pending claims is earnestly sought.

Election / Restriction

In reply to the Office Action of July 28, 2004, Applicant confirms the election of Group I of the claims, drawn to an erythropoietin serum albumin fusion protein (claims 1-26, 46 and 47). The balance of the remarks in this action will focus on the pending claims. However, Applicant expressly retains the right to file additional divisional or continuation applications covering the non-elected invention during the pendency of the current application. Early and favorable consideration of the instant claims on the merits is earnestly solicited.

Specification

The appropriate SEQ. ID. Numbers, and replacement pages are provided herein in compliance with the Examiner's concerns. Both a clean version of the amended pages, as well as a marked up version are submitted herewith as per 37 CFR § 1.125. The Applicants thank the Examiner for a continued examination on the merits and the movement toward allowability of some or all of the pending claims that the ongoing examination represents.

Drawings

The drawings are objected to as failing to identify the appropriate SEQ ID numbers. The disclosure is amended to correct any inconsistencies present and overcome the Examiner's objections to the drawings. Corrected drawings are submitted herewith. Formal drawings will be submitted upon allowance of the claims and prior to issuance.

Sequence Listing

Submitted herein is a paper copy of the revised Sequence Listing, as well as a diskette which contains a computer readable form of the revised Sequence Listing filed herewith. It is Applicant's understanding that the enclosed Sequence Listing complies with the requirements of 37 CFR §§ 1.821(f) and (g); and 1.824. The material on this diskette is identical in substance to the sequences appearing on noted pages 3-4, 6-18 and 26 of the amended specification. Furthermore, Applicant hereby states that the information recorded in computer readable form is identical to the written sequence listing filed herewith as required by § 1.824(f) and does not contain any new matter as required under 37 CFR § 1.821(g).

Double Patenting

Claims 1-26 and 46-47 are provisionally rejected under the judicially created doctrine of "Double Patenting." As stated by the Examiner, "a timely filed terminal disclaimer" filed "in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection" (Office Action of July 28, 2004, page 4). That is, a rejection based on a nonstatutory type of double patenting can be avoided by filing a terminal disclaimer in the application or proceeding in which the rejection is made. In re Vogel, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); In re Knohl, 386 F.2d 476, 155 USPQ 586 (CCPA 1967); and, In re Griswold, 365 F.2d 834, 150 USPQ 804 (CCPA 1966).

Respectfully, Applicants also point out that the filing of a Terminal Disclaimer to obviate a rejection based on nonstatutory double patenting is not an admission of the propriety of the rejection. Quad Environmental Technologies Corp. v. Union Sanitary District, 946 F.2d 870, 20 USPQ2d 1392 (Fed. Cir. 1991). In this sense the filing of a terminal disclaimer simply serves the statutory function of removing the rejection of double patenting, and raises neither a presumption nor estoppel on the merits of the rejection.

Therefore, any Disclaimer filed by Applicant would make plain the common ownership of the cited patent applications and would thereby effectively make the cited applications free of the prior art and ready for allowance. MPEP § 1504.06; MPEP § 804.02 Accordingly, upon the filing of a Notification of Allowance for the current pending claims it is Applicants' position that a Terminal Disclaimer will be filed relative to the term of co-pending application No. 10/768,873, or vice-versa. Until such time as the claims are allowed this rejection remains provisional. However, Applicants believe that the offer of filing an appropriate Terminal Disclaimer, made herein, should remove this objection to allowance of the claims.

Given the above comments, it is Applicant's position that this rejection has been overcome. Putting this objection and provisional rejection in abeyance is respectfully requested.

Rejection under 35 U.S.C. § 112, second paragraph,

Claims 1-26, 46 and 47 are rejected under 35 U.S.C. § 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter of the invention. The claims are amended to overcome the rejection.

Claims 1, 2, 24, 46 and 47 are amended to reflect the Examiner's concerns and are now believed to comply with the provisions of 35 U.S.C. § 112, second paragraph. Importantly, independent claim 1 is the base claim for claims 2-26 and with its amendment the other claims are also modified. Thus, the Examiner's rejection of these claims, based on §112, second paragraph, is overcome. Reconsideration of claims 1-26, 46 and 47 is therefore respectfully requested.

New Claims 48-68 also reflect the Examiner's concerns as voice in the most recent Office Action and mirror many of the amendments made in the previously rejected claims. Favorable consideration of the new claims is therefore respectfully requested.

The Rejections Under 35 U.S.C. §103(a)

Bill et al., Korhonen et al., and Syed et al.

Claims 1-26, 46 and 47 are rejected under 35 U.S.C. § 103(a) as being obvious over Bill et al. (BBA 1995), Bill et al., (BBA 1997), Korhonen et al., in view of Syed et al. This rejection of the claims is respectfully traversed.

The basic considerations which apply to obviousness rejections under MPEP § 2141 are as follows:

(1) the claimed invention must be considered as a whole;

(2) the references must be considered as a whole and must suggest the desirability and thus the obviousness of making the combination;

(3) the references must be viewed without the benefit of impermissible hindsight vision afforded by the claimed invention; and

(4) reasonable expectation of success is the standard by which obviousness is determined.

When the prior art itself fails to meet even one of the above criteria the cited art does not satisfy 35 U.S.C. § 103(a) and prevents the establishment of the required prima facie case of obviousness by the Examiner. In re Oetiker, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992); In re Rijckaert, 28 USPQ2d 1955, 1956 (Fed. Cir. 1993). As pointed out below, the prior art not only fails to provide the suggestion, or incentive to combine but also fails to provide any reasonable expectation of success for the piecemeal combination of the prior art into something resembling the instant invention.

Bill et al,.

Bill et al. (both references) discloses an experimental designed to express a GST fusion protein. However, there is no conception of the current EPO-hSA fusion construct or its variations in terms of peptide linker or specific glycosylation whatsoever. The molecule of Bill et al., is one built for cleavage. This a strategy clear at variance with the current claims which utilize a fusion protein construct precisely to change the pharmokinetics of the resultant protein and to increase half-life. Moreover, Applicants believe that the amendments to the current claims reciting a human albumin and a human EPO moiety with an identifiable linker section obviate any rejections based on Bill et al. For this reason these citations are simply inapposite to the currently cited and pending claims.

Syed et al.,

Syed et al. does not provide what Bill et al. lacks. Syed et al., like the Bill et al., citations discloses a variant molecule (hirudin) with variant activity. The current claims recite a human albumin, a human EPO moiety, and an essentially a synthetic peptide linkage – Syed et al., instead provides a rabbit with a variant hirudin molecule with no apparent linker molecule. Each of these elements of the current claims carries with it

purpose that is not rendered obvious by Syed et al. Essentially Syed et al., provides no information, teaching or predictive value for the prior art that is necessary to sustain an obviousness argument. The human albumin fusion of the current invention will have variant pharmokinetics, glycosylation, and steric activity. Each element of the current composition is at variance with the composition put together by Syed et al. Reconsideration is therefore respectfully requested. In addition, Syed and Bill both fail to suggest, expressly or implicitly, any need or possibility of combining their disparate teachings in such a way that they might then read on the instant claims.

Korhonen et al.,

Korhonen et al. also fails to provide what the Bill and Syed references lack. Korhonen teaches a system of making and suggests a possible method of EPO expression. However, looking at the Korhonen et al., citation it is clear that the reference is text case of a failed experiment teaching nothing to those working in the field. Moreover, some of the teachings would take those in the field in a direction at considerable variance to that of the present disclosure.

To whit, used a beta-lactoglobulin gene as the fusion partner for the production and expression of EPO. This is a construct considerably different than that recited in the instant claims. As pointed out in the specification the purpose of the current invention is to provide an EPO product with a fusion partner and variances in glycosylation with the goal of increasing half-life and providing a new species of therapeutic composition. For this purpose human albumin was deliberately chosen.

Focusing on Korhonen it not only is a fusion product designed for cleavage – a separate pathway for development of an EPO molecule but failed in doing so. Looking at the biological activity of the molecule it was less than "15% of that of human recombinant) (Korhonen et al., Abstract), directly counter any teaching that would lead any worker in the field toward a fusion protein with an EPO moiety – in fact teaching against it. Therefore high hurdle of a prima facie rejection based on obviousness to be found appropriate, the disclosure cited against a given application must express essentially the same elements and outcome of the claimed discovery. No element of significance can be missing, all must be put in the hands of the public so that they can

practice the invention, without reference to a separate applications' teachings. International Glass Co. v. United States, 408 F.2d at 402, 159 USPQ at 440 (1966). Thus, in order to function as an obviousness reference under 35 U.S.C. §103(a) it is necessary that the cited reference must teach some element of the invention as a whole, and not teach away from it. As the United States Supreme Court has stated, "An inoperable invention or one which fails to achieve its intended result does not negative novelty." United States v. Adams, 383 U.S. 39 at 49-51 (1966). For this reason, failed experiments or inoperative inventions, such as those catalogued by Korhonen et al., cannot be considered prior art sufficient to support an Examiner's rejection, be it one based on anticipation or obviousness. In re Wilder, 429 F.2d 447, 166 USPQ 545 at 548 (CCPA 1970). For this reason Applicants believe that Korhonen teaches against the current invention and is therefore unavailable for combination.

It is therefore respectfully suggested that claims 1-26, 46 and 47are not obvious over the combination of Bill et al., Syed et al. and Korhonen et al. The dependent claims being dependent upon and further limiting independent claim 1, should also be allowable for that reason, as well as for the additional recitations they contain. Applicants respectfully request reconsideration of the rejection of claims 1-26, 46 and 47 under 35 U.S.C. § 103(a) in view of the above amendments and remarks.

New claims 48-68 also fall within the arguments made above. Favorable consideration is respectfully requested.

Applicants respectfully submit that the pending claims of this application are in condition for allowance, and that this case is now in condition for allowance of all claims therein. Such action is thus respectfully requested.

If the Examiner disagrees with any of the remarks herein, or believes for any other reason that direct contact with Applicant's attorney would advance the prosecution of the case to finality, the Examiner is invited to telephone the undersigned at the number given below. Consideration of this Amendment is respectfully requested.

The Commissioner is authorized to charge any fee which may now or hereafter be due for this divisional application to GTC Biotherapeutics' Deposit Account No. 502092.

Early and favorable action is earnestly solicited.

Date: 1/28/04

Respectfully Submitted,

By: _____

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acids in the peptide linker is selected from the group consisting of (Gly, Ser, Asn, Thr and Ala; the peptide linker includes a Gly-Ser element.

In a preferred embodiment, the fusion protein includes a peptide linker and the peptide linker includes a sequence having the formula (Ser-Gly-G1y-G1y-G1y)y (SEQ. ID 1) wherein y is 1, 2,3, 4, 5, 6,7, or 8. Preferably, the peptide linker includes a sequence having the formula $(Ser\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Gly)_3$ (SEQ. ID 1). Preferably, the peptide linker includes a sequence having the formula $((Ser\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Gly_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3).

In a preferred embodiment, the fusion protein includes a peptide linker and the peptide linker includes a sequence having the formula (Ser-Ser-Ser-Ser-Gly)y (SEQ. ID 4) wherein y is 1,2, 3, 4, 5,6, 7, or 8. Preferably, the peptide linker includes a sequence having the formula $((Ser\text{-}Ser\text{-}Ser\text{-}Ser\text{-}Gly)_3\text{-}Ser\text{-}Pro)$ (SEQ. ID 4).

In another aspect, the invention features, an EPOa-hSA fusion protein wherein the EPOa includes amino acid residues G1n24, G1n38, G1n83 and A1a126.

In a preferred embodiment the EPOa is G1n24, 01n38, Gln83, A1a126 EPO (i.e., only amino acids 24, 38, 83, and 126 differ from wild type).

In another aspect, the invention features, an EPOa-hSA fusion protein which includes from left to right, an EPOa which includes amino acid residues G1n24, Gln38, G1n83 and Ala126, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-Gly-GIy-Gly)$_4$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the EPOa is G1n24, G1n38, G1n83, A1a126 EPO.

In a preferred embodiment the fusion protein is from left to right, G1n24, G1n38, Gln83, Ala126 EPO, a peptide linker having the formula $((Ser\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and human serum albumin.

In another aspect, the invention features, an EPOa-hSA fusion protein which includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula $((Ser\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and an EPOa which includes amino acid residues G1n24, G1n38, G1n83 and Ala126.

In a preferred embodiment the EPOa is G1n24, G1n38, Gln83, Ala126 EPO.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and Gln24, Gln38, G1n83, A1a126 EPO.

In another aspect, the invention features, an isolated nucleic acid having a nucleotide sequence which encodes an EPOa-hSA fusion protein described herein, e.g., an EPOa-hSA fusion protein wherein at least one amino acid residue is altered such that a site which serves as a site for glycosylation in EPO does not serve as a site for glycosylation in the EPOa, e.g., an EPOa-hSA fusion protein in which at least one amino acid residue of the encoded EPOa-hSA which can serve as a glycosylation site in erythropoietin is altered, e.g., by substitution or deletion, such that it does not serve as a glycosylation site.

In another aspect, the invention features, a nucleic acid which encodes an EPOa-hSA fusion protein wherein the EPOa includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In another aspect, the invention features, a nucleic acid which encodes an EPOa-hSA fusion protein which includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and human serum albumin.

In another aspect, the invention features, a nucleic acid which encodes an EPOa-hSA fusion protein which includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

In another aspect, the invention features, an expression vector or a construct which includes a nucleic acid of the invention.

linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[$_3$]$_4$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$_4$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment, the EPOa-hSA fusion protein includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$_4$-Ser-Pro) (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$_4$-Ser-Pro) (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

The invention also includes a cultured cell which includes a nucleic acid which encodes an EPOa-hSA fusion protein, e.g., an EPOa-hSA fusion protein described herein. The invention also includes methods of making such cells, e.g., by introducing into the cell, or forming in the cell, a nucleic acid which encodes an EPOa-hSA fusion protein, e.g., an EPOa-hSA fusion protein described herein.

In another aspect, the invention features, a method of making an EPOa-hSA fusion protein, e.g., an EPOa-hSA described herein. The method includes providing a transgenic organism which includes a transgene which directs the expression of EPOa-hSA fusion protein; allowing the transgene to be expressed; and, preferably, recovering a transgenically produced EPOa-hSA fusion protein, e.g., from the organism or from a product produced by the organism.

In a preferred embodiment, the transgenic organism is a transgenic animal, e.g., a transgenic mammal, e.g., a transgenic dairy animal, e.g., a transgenic goat or a transgenic cow.

In a preferred embodiment, the EPOa-hSA fusion protein is secreted into a bodily fluid and the method further includes purifying the EPOa-hSA fusion protein from the bodily fluid.

In a preferred embodiment, the transgenically produced EPOa-hSA fusion protein is made in a mammary gland of a transgenic mammal, preferably under the control of a milk specific promoter, e.g., a milk serum protein or casein promoter. The milk specific

promoter can be a casein promoter, beta lactoglobulin promoter, whey acid protein promoter, or lactalbumin promoter. Preferably, the promoter is a goat β casein promoter.

In preferred embodiments, the EPOa-hSA fusion protein is made in a mammary gland of the transgenic mammal, e.g., a ruminant, e.g., a dairy animal, e.g., a goat or cow.

In preferred embodiments, the EPOa-hSA fusion protein is secreted into the milk of a transgenic mammal at concentrations of at least about 0.2 mg/ml, 0.5 mg/ml, 0.75 mg/ml, 1 mg/ml, 2 mg/ml, 3 mg/ml or higher.

In preferred embodiments the method further includes recovering EPOa-hSA fusion protein from the organism or from a product produced by the organism, e.g., milk, seeds, hair, blood, eggs, or urine.

In yet another embodiment, the EPOa-hSA fusion protein is produced in a transgenic plant.

In a preferred embodiment, the erythropoietin analog includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment, the EPOa-hSA fusion protein includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and human serum albumin.

In a preferred embodiment, the EPOa-hSA fusion protein includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3),and Gln24, Gln38, Gln83, Ala126 EPO.

In another aspect, the invention features, a method of making a transgenic EPOa-hSA fusion protein, e.g., an EPOa-hSA fusion described herein. The method includes providing a transgenic animal, e.g., goat or a cow, which includes a transgene which provides for the expression of the EPOa-hSA fusion protein; allowing the transgene to be

expressed; and, preferably, recovering EPOa-hSA fusion protein, from the milk of the transgenic animal.

In preferred embodiments, the EPOa-hSA fusion protein is made in a mammary gland of the transgenic mammal, e.g., a ruminant, e.g., a goat or a cow.

In preferred embodiments, the EPOa-hSA fusion protein is secreted into the milk of the transgenic mammal, e.g., a ruminant, e.g., a dairy animal, e.g., a goat or a cow.

In preferred embodiments, the EPOa-hSA fusion protein is made under the control of a mammary gland specific promoter, e.g., a milk specific promoter, e.g., a milk serum protein or casein promoter. The milk specific promoter can be a casein promoter, beta lactoglobulin promoter, whey acid protein promoter, or lactalbumin promoter. Preferably, the promoter is a goat β casein promoter.

In preferred embodiments, the EPOa-hSA fusion protein is secreted into the milk of a transgenic mammal at concentrations of at least about 0.2 mg/ml, 0.5 mg/ml, 0.75 mg/ml, 1 mg/ml, 2 mg/ml, 3 mg/ml or higher.

In a preferred embodiment, the EPOa includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment, the EPOa-hSA fusion protein includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$_4$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$_4$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment, the EPOa-hSA fusion protein includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)[$_3$]$_4$-Ser-Pro) (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$_4$-Ser-Pro) (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

In another aspect, the invention features, a method for providing a transgenic preparation which includes an EPOa-hSA fusion protein, e.g., an EPOa-hSA fusion protein

described herein, in the milk of a transgenic mammal. The method includes: providing a transgenic mammal having an EPOa-hSA fusion protein protein-coding sequence operatively linked to a promoter sequence that results in the expression of the protein-coding sequence in mammary gland epithelial cells, allowing the fusion protein to be expressed, and obtaining milk from the mammal, thereby providing the transgenic preparation.

In a preferred embodiment, the EPOa-hSA fusion protein-coding sequence operatively linked to a promoter sequence is introduced into the germline of the transgenic mammal.

In a preferred embodiment, the erythropoietin analog includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment, the EPOa-hSA fusion protein includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$_4$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$_4$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment, the EPOa-hSA fusion protein includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$_4$-Ser-Pro) (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$_4$-Ser-Pro) (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

In another aspect, the invention features, a method for providing a transgenic preparation which includes an EPOa-hSA fusion protein, e.g., an EPOa-hSA fusion protein described herein, in the milk of a transgenic goat or transgenic cow. The method includes providing a transgenic goat or cow having an EPOa-hSA fusion protein-coding sequence operatively linked to a promoter sequence that results in the expression of the protein-coding sequence in mammary gland epithelial cells, allowing the fusion protein to be

expressed, and obtaining milk from the goat or cow, thereby providing the transgenic preparation.

In a preferred embodiment, the erythropoietin analog includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment, the EPOa-hSA fusion protein includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_4$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$[_3]_4$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment, the EPOa-hSA fusion protein includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$_4$-Ser-Pro) (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$_4$-Ser-Pro) (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

In another aspect, the invention features, a transgenic organism, which includes a transgene which encodes an EPOa-hSA fusion protein, e.g., an EPOa-hSA fusion protein described herein.

In a preferred embodiment, the transgenic organism is a transgenic plant or animal. Preferred transgenic animals include: mammals; birds; reptiles; marsupials; and amphibians. Suitable mammals include: ruminants; ungulates; domesticated mammals; and dairy animals. Particularly preferred animals include: mice, goats, sheep, camels, rabbits, cows, pigs, horses, oxen, and llamas. Suitable birds include chickens, geese, and turkeys. Where the transgenic protein is secreted into the milk of a transgenic animal, the animal should be able to produce at least 1, and more preferably at least 10, or 100, liters of milk per year.

In preferred embodiments, the EPOa-hSA fusion protein is under the control of a mammary gland specific promoter, e.g., a milk specific promoter, e.g., a milk serum protein or casein promoter. The milk specific promoter can be a casein promoter, beta lactoglobulin

promoter, whey acid protein promoter, or lactalbumin promoter. Preferably, the promoter is a goat β casein promoter.

In preferred embodiments, the EPOa-hSA fusion protein is secreted into the milk at concentrations of at least about 0.2 mg/ml, 0.5 mg/ml, 0.75 mg/ml, 1 mg/ml, 2 mg/ml, 3 mg/ml or higher.

In a preferred embodiment, the EPOa includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment, the EPOa-hSA fusion protein includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and human serum albumin.

In a preferred embodiment, the EPOa-hSA fusion protein includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

In another aspect, the invention features, a transgenic cow, goat or sheep, which includes a transgene which encodes an EPOa-hSA fusion protein, e.g., an EPOa-hSA fusion protein described herein.

In preferred embodiments, the EPOa-hSA fusion protein is under the control of a mammary gland specific promoter, e.g., a milk specific promoter, e.g., a milk serum protein or casein promoter. The milk specific promoter can be a casein promoter, beta lactoglobulin promoter, whey acid protein promoter, or lactalbumin promoter. Preferably, the promoter is a goat β casein promoter.

In preferred embodiments, the EPOa-hSA fusion protein is secreted into the milk at concentrations of at least about 0.2 mg/ml, 0.5 mg/ml, 0.75 mg/ml, 1 mg/ml, 2 mg/ml, 3 mg/ml or higher.

In a preferred embodiment, the EPOa includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment, the EPOa-hSA fusion protein includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and human serum albumin.

In a preferred embodiment, the EPOa-hSA fusion protein includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

In another aspect, the invention features, a herd of transgenic animals having at least one female and one male transgenic animal, wherein each animal includes an EPOa-hSA fusion protein transgene, e.g., a transgene which encodes an EPOa-hSA fusion protein described herein.

In a preferred embodiment, a transgenic animal of the herd is a mammal, bird, reptile, marsupial or amphibian. Suitable mammals include: ruminants; ungulates; domesticated mammals; and dairy animals. Particularly preferred animals include: mice, goats, sheep, camels, rabbits, cows, pigs, horses, oxen, and llamas. Suitable birds include chickens, geese, and turkeys. Where the transgenic protein is secreted into the milk of a transgenic animal, the animal should be able to produce at least 1, and more preferably at least 10, or 100, liters of milk per year.

In preferred embodiments, the EPOa-hSA fusion protein is under the control of a mammary gland specific promoter, e.g., a milk specific promoter, e.g., a milk serum protein

or casein promoter. The milk specific promoter can is a casein promoter, beta lactoglobulin promoter, whey acid protein promoter, or lactalbumin promoter.

In preferred embodiments, the EPOa-hSA fusion protein is secreted into the milk at concentrations of at least about 0.2 mg/ml, 0.5 mg/ml, 0.75 mg/ml, 1 mg/ml, 2 mg/ml, 3 mg/ml or higher.

In a preferred embodiment, the EPOa includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment, the EPOa-hSA fusion protein includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and human serum albumin.

In a preferred embodiment, the EPOa-hSA fusion protein includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

In another aspect, the invention features, a pharmaceutical composition having a therapeutically effective amount of an EPOa-hSA fusion protein, e.g., an EPOa-hSA fusion protein described herein, and a pharmaceutically acceptable carrier.

In a preferred embodiment, the composition includes milk.

In a preferred embodiment, the EPOa includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment, the EPOa-hSA fusion protein includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$_4$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment, the EPOa-hSA fusion protein includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$_4$-Ser-Pro) (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$_4$-Ser-Pro) (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

In another aspect, the invention features, a kit having an EPOa-hSA fusion protein, e.g., an EPOa-hSA fusion protein described herein, packaged with instructions for treating a subject in need of erythropoietin.

In a preferred embodiment, the subject is a patient suffering from anemia associated with renal failure, chronic disease, HIV infection, blood loss or cancer.

In another preferred embodiment, the subject is a preoperative patient.

In a preferred embodiment, the erythropoietin analog includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment, the EPOa-hSA fusion protein includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$_4$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$_4$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment, the EPOa-hSA fusion protein includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$_4$-Ser-Pro) (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$_4$-Ser-Pro) (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

In another aspect, the invention features, a purified preparation of an EPOa-hSA fusion protein, e.g., an EPO-hSA fusion protein described herein.

In preferred embodiments, the preparation includes at least 1, 10, 100 or 1000 micrograms of EPOa-hSA fusion protein. In preferred embodiments, the preparation includes at least 1, 10, 100 or 1000 milligrams of EPOa-hSA fusion protein.

In another aspect, the invention features, an EPOa-hSA fusion protein, or a purified preparation thereof, wherein the erythropoietin analog includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In preferred embodiments, the preparation includes at least 1, 10, 100 or 1000 micrograms of EPOa-hSA fusion protein. In preferred embodiments, the preparation includes at least 1, 10, 100 or 1000 milligrams of EPOa-hSA fusion protein.

In another aspect, the invention features, an EPOa-hSA fusion protein, or a purified preparation thereof, which includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3),and human serum albumin.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)[_3]_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and human serum albumin.

In preferred embodiments, the preparation includes at least 1, 10, 100 or 1000 micrograms of EPOa-hSA fusion protein. In preferred embodiments, the preparation includes at least 1, 10, 100 or 1000 milligrams of EPOa-hSA fusion protein.

In another aspect, the invention features, an EPOa-hSA fusion protein, or a purified preparation thereof, which includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$_4$-Ser-Pro) (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

In preferred embodiments, the preparation includes at least 1, 10, or 100 milligrams of EPOa-hSA fusion protein. In preferred embodiments, the preparation includes at least 1, 10, or 100 grams of EPOa-hSA fusion protein.

In another aspect, the invention features, a method of treating a subject, e.g., a human, in need of erythropoietin. The method includes administering a therapeutically effective amount of an EPOa-hSA fusion protein, e.g., an EPO-hSA fusion protein described herein, to the subject.

In a preferred embodiment, the subject is a patient suffering from anemia associated with renal failure, chronic disease, HIV infection, blood loss or cancer.

In another preferred embodiment, the subject is a preoperative patient.

In preferred embodiments the EPOa-hSA is administered repeatedly, e.g., at least two, three, five, or 10 times.

In a preferred embodiment, the erythropoietin analog includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment, the EPOa-hSA fusion protein includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$_4$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$_4$-Ser-Pro) (SEQ. ID 3), and human serum albumin.

In a preferred embodiment, the EPOa-hSA fusion protein includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$_4$-Ser-Pro) (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula ((Ser-Gly-G1y-GIy-Gly)$_4$-Ser-Pro) (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

In another aspect, the invention features, a method of treating a subject in need of erythropoietin. The method includes delivering or providing a nucleic acid encoding an EPOa-hSA fusion protein, e.g., a fusion protein described herein, to the subject.

In a preferred embodiment, the nucleic acid is delivered to a target cell of the subject.

In a preferred embodiment, the nucleic acid is delivered or provided in a biologically effective carrier, e.g., an expression vector.

In a preferred embodiment, the nucleic acid is delivered or provided in a cell, e.g., an autologous, allogeneic, or xenogeneic cell.

In a preferred embodiment, the EPOa includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment, the EPOa-hSA fusion protein includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and human serum albumin.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and human serum albumin.

In a preferred embodiment, the EPOa-hSA fusion protein includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

In another aspect, the invention features, a method of making a transgenic organism which has an EPOa-hSA transgene. The method includes providing or forming in a cell of an organism, an EPOa-hSA transgene, e.g., a transgene which encodes an EPOa-hSA fusion protein described herein; and allowing the cell, or a descendent of the cell, to give rise to a transgenic organism.

In a preferred embodiment, the transgenic organism is a transgenic plant or animal. Preferred transgenic animals include: mammals; birds; reptiles; marsupials; and

amphibians. Suitable mammals include: ruminants; ungulates; domesticated mammals; and dairy animals. Particularly preferred animals include: mice, goats, sheep, camels, rabbits, cows, pigs, horses, oxen, and llamas. Suitable birds include chickens, geese, and turkeys. Where the transgenic protein is secreted into the milk of a transgenic animal, the animal should be able to produce at least 1, and more preferably at least 10, or 100, liters of milk per year.

In preferred embodiments, the EPOa-hSA fusion protein is under the control of a mammary gland specific promoter, e.g., a milk specific promoter, e.g., a milk serum protein or casein promoter. The milk specific promoter can be a casein promoter, beta lactoglobulin promoter, whey acid protein promoter, or lactalbumin promoter. Preferably, the promoter is a goat β casein promoter.

In preferred embodiments, the organism is a mammal, and the EPOa-hSA fusion protein is secreted into the milk of the transgenic animal at concentrations of at least about 0.2 mg/ml, 0.5 mg/ml, 0.75 mg/ml, 1 mg/ml, 2 mg/ml, 3 mg/ml or higher.

In a preferred embodiment, the EPOa includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the EPOa is Gln24, Gln38, Gln83, Ala126 EPO.

In a preferred embodiment, the EPOa-hSA fusion protein includes from left to right, an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126, a peptide linker, e.g., a peptide linker having the formula $((Ser\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Gly)_3\text{-}Ser\text{-}Pro)$, and human serum albumin.

In a preferred embodiment the fusion protein is from left to right, Gln24, Gln38, Gln83, Ala126 EPO, a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and human serum albumin.

In a preferred embodiment, the EPOa-hSA fusion protein includes, from left to right, human serum albumin, a peptide linker, e.g., a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and an EPOa which includes amino acid residues Gln24, Gln38, Gln83 and Ala126.

In a preferred embodiment the fusion protein is from left to right, human serum albumin, a peptide linker having the formula $((Ser\text{-}Gly\text{-}G1y\text{-}GIy\text{-}Gly)_4\text{-}Ser\text{-}Pro)$ (SEQ. ID 3), and Gln24, Gln38, Gln83, Ala126 EPO.

advantageously from about 10 to about 20 amino acids. Amino acid sequences useful as linkers of EPOa and hSA include, but are not limited to, $(SerGly_4)_y$ (SEQ ID 1) wherein y is greater than or equal to 8, or $Gly_4SerGly_5Ser$ (SEQ ID 2). A preferred linker sequence has the formula $(SerGly_4)_4$ (SEQ ID 1). Another preferred linker has the sequence ((Ser-Ser-Ser-Ser-Gly)$_3$-Ser-Pro) (SEQ ID 4).

The EPOa and hSA proteins can be directly fused without a linker sequence. Linker sequences are unnecessary where the proteins being fused have non-essential N-or C-terminal amino acid regions which can be used to separate the functional domains and prevent steric interference. In preferred embodiments, the C-terminus of EPOa can be directly fused to the N-terminus of hSA or the C-terminus of hSA can be directly fused to the N-terminus of EPOa.

Recombinant Production

An EPOa-hSA fusion protein can be prepared with standard recombinant DNA techniques using a nucleic acid molecule encoding the fusion protein. A nucleotide sequence encoding a fusion protein can be synthesized by standard DNA synthesis methods.

A nucleic acid encoding a fusion protein can be introduced into a host cell, e.g., a cell of a primary or immortalized cell line. The recombinant cells can be used to produce the fusion protein. A nucleic acid encoding a fusion protein can be introduced into a host cell, e.g., by homologous recombination. In most cases, a nucleic acid encoding the EPOa-hSA fusion protein is incorporated into a recombinant expression vector.

The nucleotide sequence encoding a fusion protein can be operatively linked to one or more regulatory sequences, selected on the basis of the host cells to be used for expression. The term "operably linked" means that the sequences encoding the fusion protein compound are linked to the regulatory sequence(s) in a manner that allows for expression of the fusion protein. The term "regulatory sequence" refers to promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990), the content of which are incorporated herein by reference. Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cells, those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences) and those that direct expression in a regulatable manner (*e.g.*, only in the presence of an inducing agent). It will be appreciated by those skilled in the art that the design of the expression vector may depend on such factors as the choice of the host cell to